Sekundær farmakologi i legemiddelutvikling:

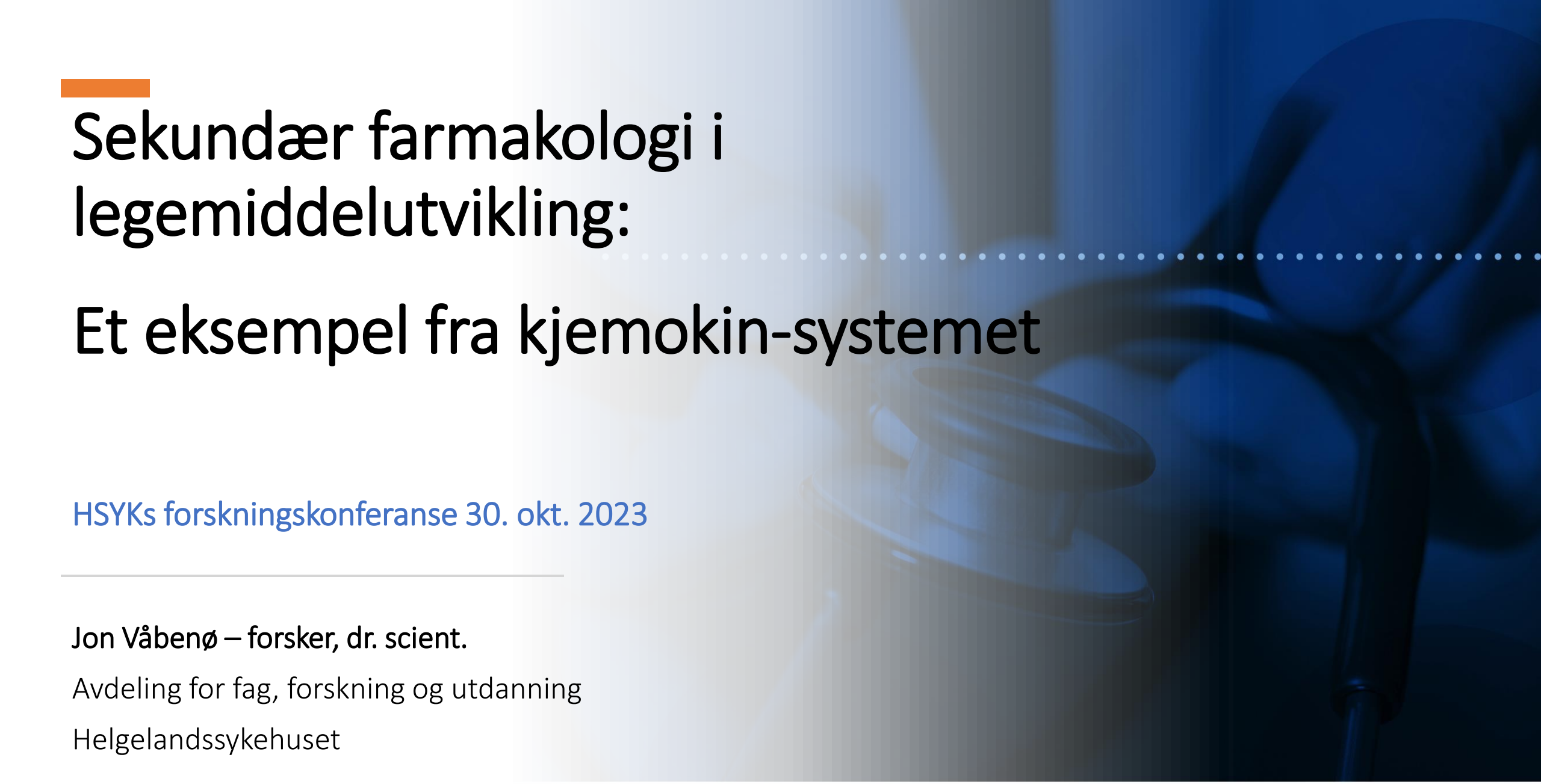
Et eksempel fra kjemokin-systemet

HSYKs forskningskonferanse 30. okt. 2023

Jon Våbenø – forsker, dr. scient.

Avdeling for fag, forskning og utdanning

Helgelandssykehuset



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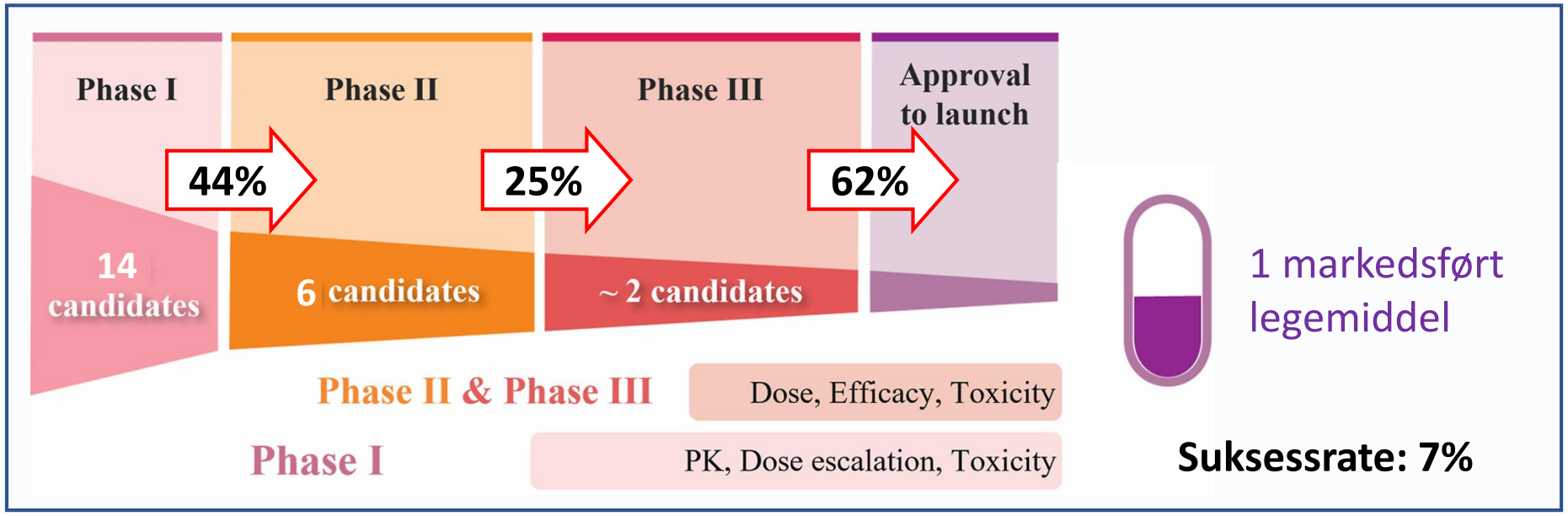
HELGELAANTEN SKIEMTJE-GÅETIE

13 av 14 kliniske legemiddelutprøvinger feiler



Dowden & Munro, *Nat. Rev. Drug Disc.* 2019

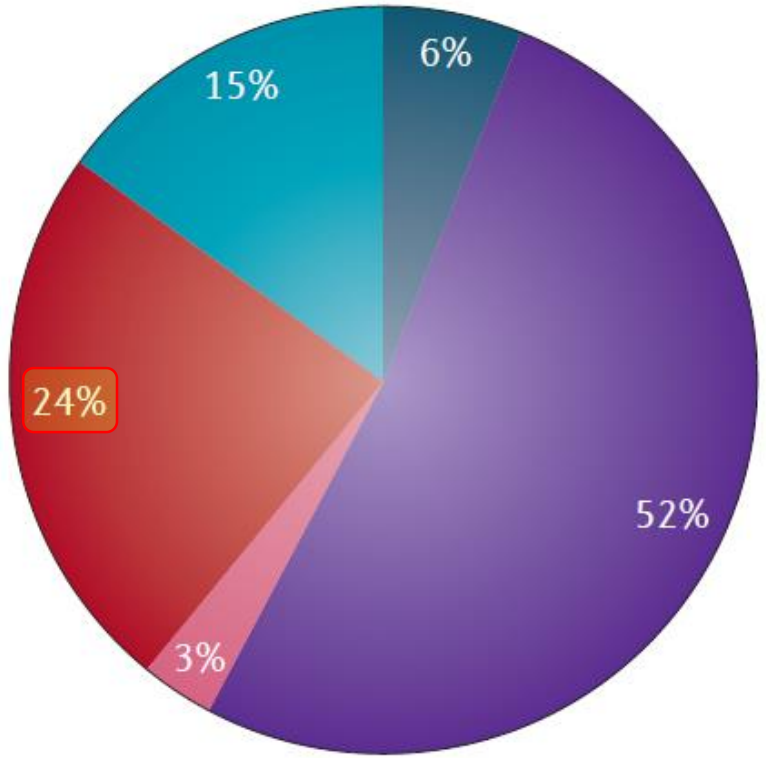
Hvorfor feiler kliniske legemiddelutprøvinger?

Vitenskapelige årsaker (ca ¾)

- Efficacy
  - Ikke tilstrekkelig terapeutisk effekt
- Safety
  - For omfattende bivirkninger og/eller toksiske effekter

Andre årsaker (ca ¼)

- Strategisk
- Kommersielt
- Operasjonelt/teknisk



Harrison, *Nat. Rev. Drug Disc.* 2016

AstraZeneca: The 5R Framework

Right target

- Strong link between target and disease
- Differentiated efficacy
- Available and predictive biomarkers

Right tissue

- Adequate bioavailability and tissue exposure
- Definition of PD biomarkers
- Clear understanding of preclinical and clinical PK/PD
- Understanding of drug–drug interactions

Right safety

- Differentiated and clear safety margins
- Understanding of secondary pharmacology risk spesifisitet/selektivitet for target
- Understanding of reactive metabolites, genotoxicity and drug–drug interactions
- Understanding of target liability

Right patient

- Identification of the most responsive patient population
- Definition of risk–benefit for a given population

Right commercial potential

- Differentiated value proposition versus future standard of care
- Focus on market access, payer and provider
- Personalized health-care strategy, including diagnostics and biomarkers

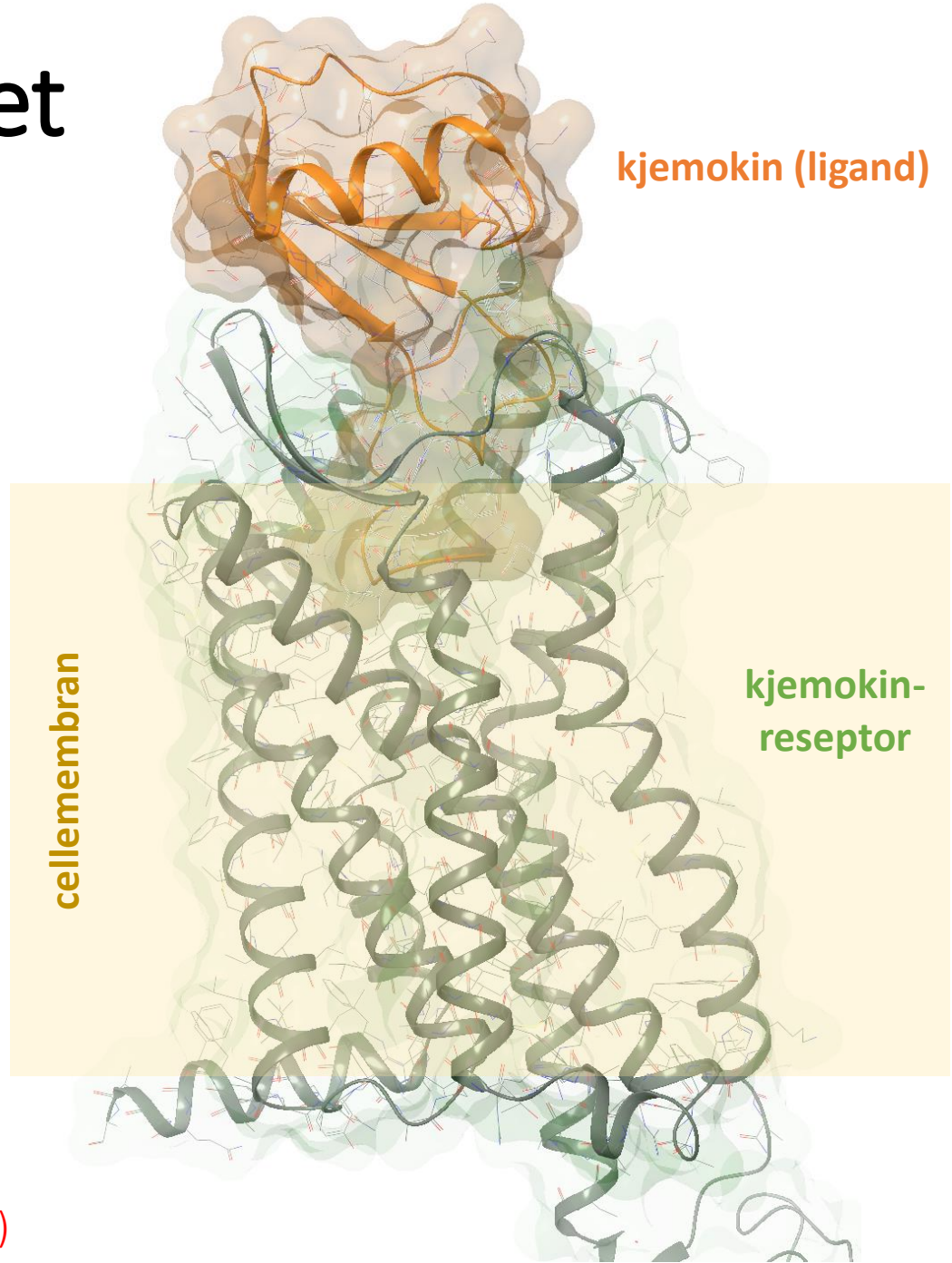
Impact of a five-dimensional framework on R&D productivity at AstraZeneca

Paul Morgan, Dean G. Brown, Simon Lennard, Mark J. Anderton, J. Carl Barrett, Ulf Eriksson, Mark Fidock, Bengt Hamrén, Anthony Johnson, Ruth E. March, James Matcham, Jerome Mettetal, David J. Nicholls, Stefan Platz, Steve Rees, Michael A. Snowden and Menelas N. Pangalos

Nat. Rev. Drug Disc. **2018**

Relevans for kjemokin-systemet

- Kjemokin = **kjemo**taktisk cyto**kin**
- En proteinfamilie med ca. 50 medlemmer; opprinnelig identifisert som mediatorer for retningsbestemt migrering (kjemotakse) av immunceller til inflammasjons-/skadested
- Binder til egne kjemokin-reseptorer på målceller; disse utgjør en underfamilie av G protein-koblede reseptorer (GPCRs)
- Finnes også virale kjemokiner/reseptorer
- Kjemokin-systemet utgjør relevante targets for:
  - Inflammatoriske/immunologiske sykdommer
  - Kreftsykdommer
  - Virusinfeksjoner (inkl. HIV)



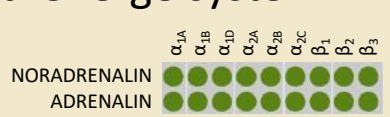
Dekket i tidligere HSYK-forelesninger: https://youtu.be/lRqntI4tSvA (2021)
https://youtu.be/E2l775pG-hY (2022)

Farmakologiske utfordringer

Svært komplekst system

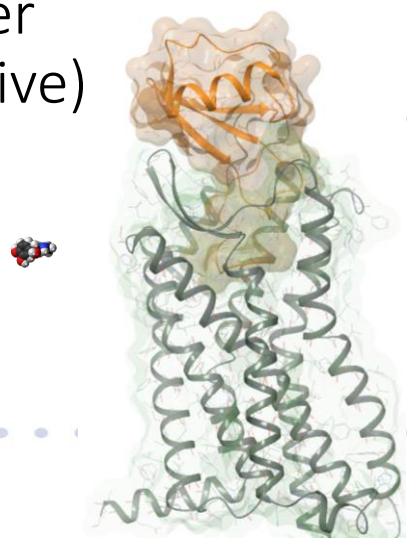
- 50+ kjemokiner x 33+ reseptorer = **1650+** mulige kombinasjoner

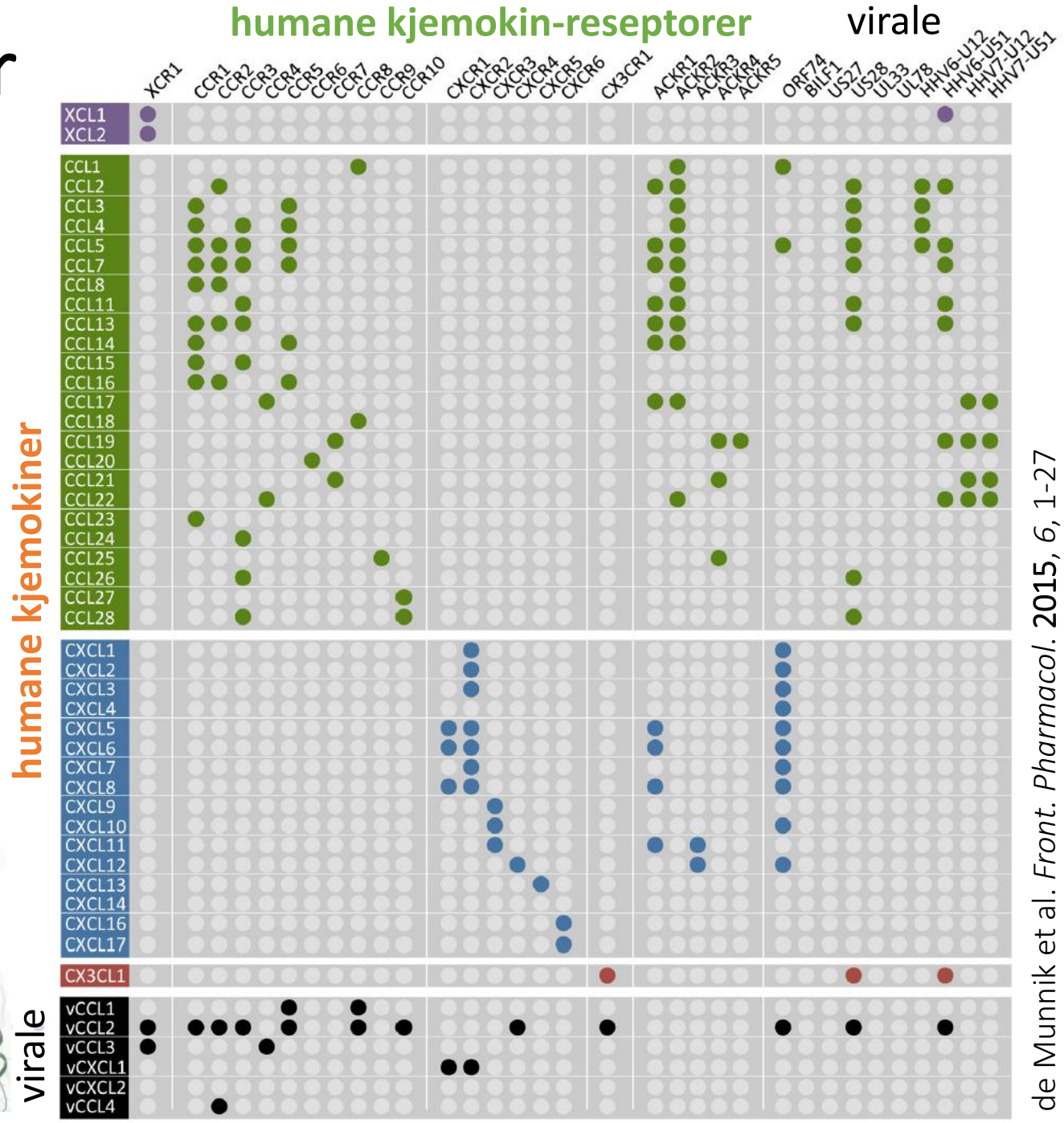
Til sammenligning: Det adrenerge system
- 2 kroppsegne ligander
- 9 reseptorer



Sekundær farmakologi (safety)

- Utvikle små molekyler som er spesifikke (eller svært selektive) for én reseptor

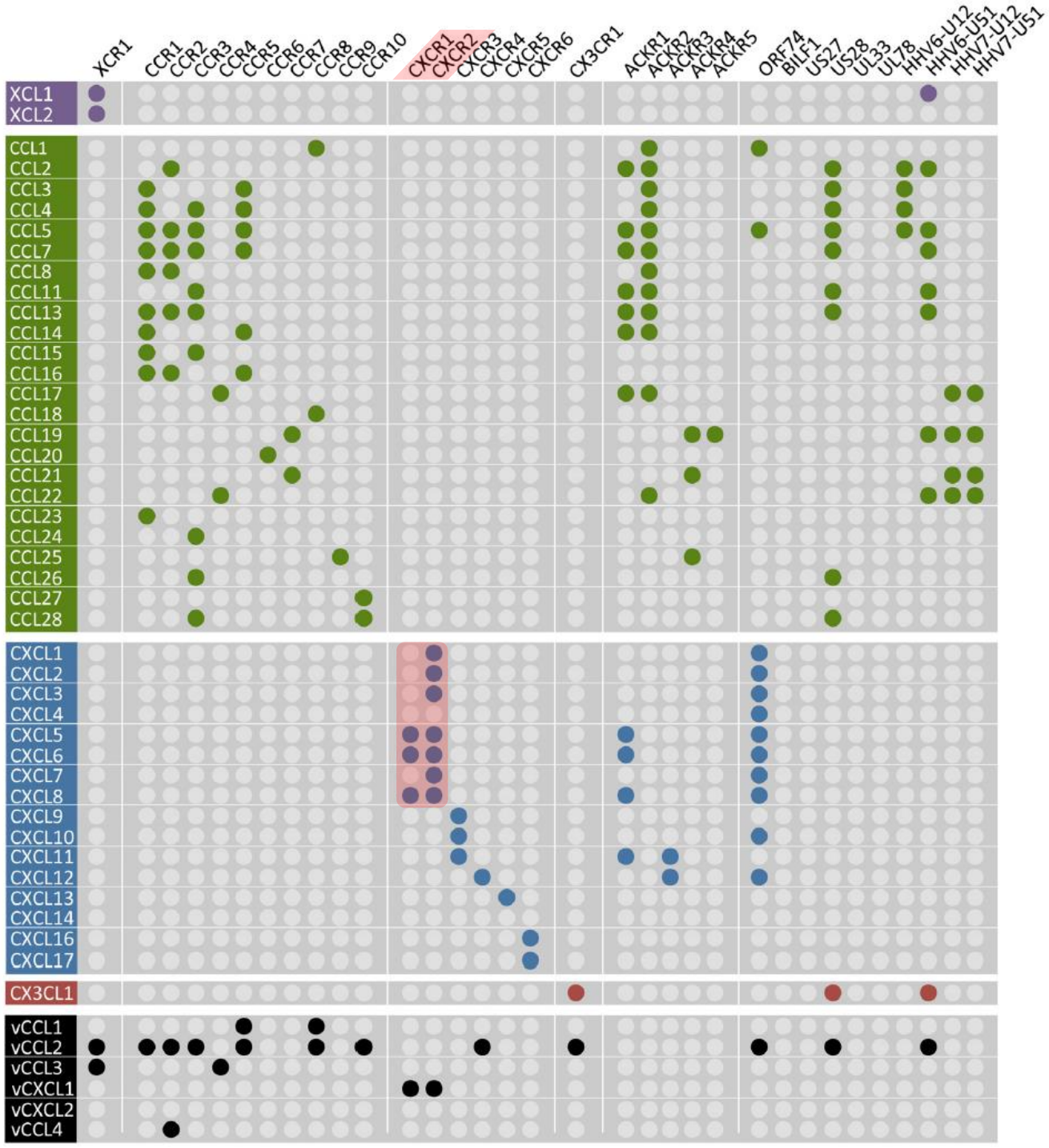




de Munnik et al. *Front. Pharmacol.* **2015**, *6*, 1-27

CXCR1 og CXCR2

- Reseptorene CXCR1 og CXCR2 er begge interessante drug targets
- CXCR1 og CXCR2 har svært like aminosyre-sekvenser (77%), og delvis overlappende kjemokin-profil
- Reseptor-spesifisitet er en stor utfordring
- Flere feilede kliniske utprøvinger utført på antagonister for CXCR1/2



Kjemokin-systemet: Kliniske legemiddelutprøvinger

Table 1 (utdrag)
Chemokine receptor drugs launched, in clinical trials or terminated.

Therapeutic indication	Drug target	Drug name	Company	Clinical phase	Activity
HIV infection	CCR5	Maraviroc	Pfizer	Approved	Launched
Hematopoeitic stem cell mobilization	CXCR4	Plerixafor (Mozobil/ AMD3100)	Genzyme	Approved	Launched
T-cell lymphoma (and allergic diseases)	CCR4	Mogamulizumab (KW-0761)	Amgen/Kyowa-Hakko	Approved	Launched
HIV infection	CCR5	Vicriviroc	Schering-Plough	III	Terminated
Cröhn's disease, celiac disease	CCR9	CCX282 (Traficet)	Chemocentryx	III	Ongoing
Asthma	CCR3 (and IL-3/IL-5/GMCSF)	ASM8	Pharmaxis	II	Ongoing
Asthma and allergic rhinitis	CCR3	GSK766994	GSK	II	Terminated
Asthma	CCR3	GSK766904	GSK	II	Terminated
Allergic rhinitis	CCR3	AZD3778	Astra Zeneca	II	Terminated
Transplant rejection, reperfusion injury	CXCR1/2	Reparixin	Dompe	II	Terminated
COPD	CXCR2	SCH 527123	Schering-Plough	II	Terminated
COPD, asthma, psoriasis	CXCR2	SCH-527123	Pharmacopeia	II	Terminated
HIV infection	CXCR4	AMD-070	AnorMed	II	Terminated
Rheumatoid arthritis	CCR1	MLN-3897	Millennium	II	Terminated

Reparixin: antagonist for CXCR1/2 (CXCR1-selektiv)
SCH 527123: antagonist for CXCR2/1 (CXCR2-selektiv)

Identifisering av funksjonelle forskjeller mellom CXCR1 og CXCR2 kan bidra til utvikling av mer spesifikke ligander/legemidler!

Solari et al. *Eur. J. Pharmacol.* **2015**, *746*, 363-367

Paper

ACS Pharmacology & Translational Science

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Letter

Identification of a Salt Bridge That Is Functionally Important for Chemokine Receptor CXCR1 but not CXCR2

Published as part of the ACS Pharmacology & Translational Science virtual special issue "GPCR Signaling".

Jon Våbenø,* Marta Oliva-Santiago, Astrid S. Jørgensen, Stefanie Karlshøj, and Mette M. Rosenkilde*

Cite This: *ACS Pharmacol. Transl. Sci.* 2023, 6, 1120−1128

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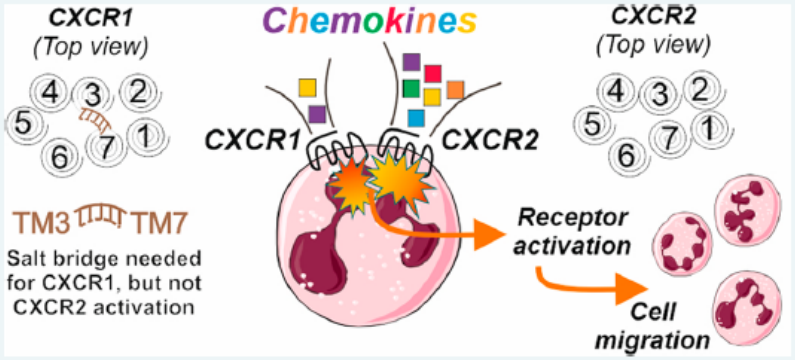
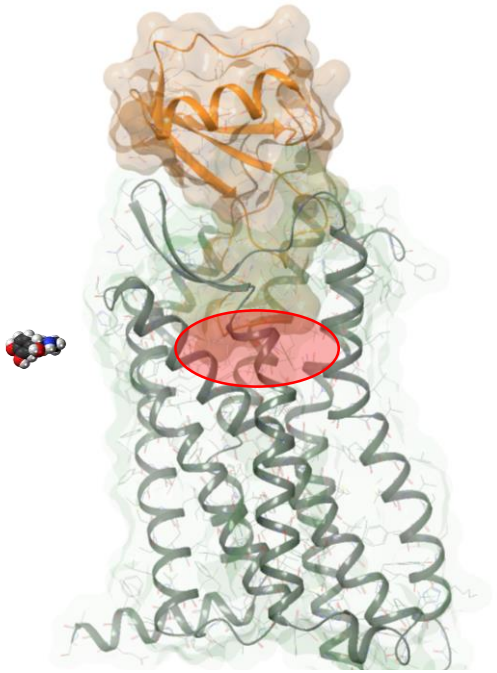
ABSTRACT: CXC chemokine receptors 1 (CXCR1) and 2 (CXCR2) have high sequence similarity and overlapping chemokine ligand profiles. Residue positions 3.32 and 7.39 are critical for signal transduction in the related CXCR4, and in these positions CXCR1 and CXCR2 contain oppositely charged residues ($Lys^{3.32}$ and $Glu^{7.39}$). Experimental and computed receptor structures reveal the possible formation of a salt bridge between transmembrane (TM) helices 3 and 7 via these two residues. To investigate the functional importance of $Lys117^{3.32}$ and $Glu291^{7.39}$ in CXCR1, along with the flanking $Glu118^{3.33}$, we performed a signaling study on 16 CXCR1 mutants using two different CXCL8 isoforms. While single Ala-mutation ($K117^{3.32}A$, $E291^{7.39}A$) and charge reversal ($K117^{3.32}E$, $E291^{7.39}K$) resulted in nonfunctional receptors, double ($K117^{3.32}E$-$E291^{7.39}K$) and triple ($K117^{3.32}E$-$E118^{3.33}A$-$E291^{7.39}K$) mutants rescued CXCR1 function. In contrast, the corresponding mutations did not affect the CXCR2 function to the same extent. Our findings show that the $Lys^{3.32}$-$Glu^{7.39}$ salt bridge between TM3 and −7 is functionally important for CXCR1 but not for CXCR2, meaning that signal transduction for these highly homologous receptors is not conserved.

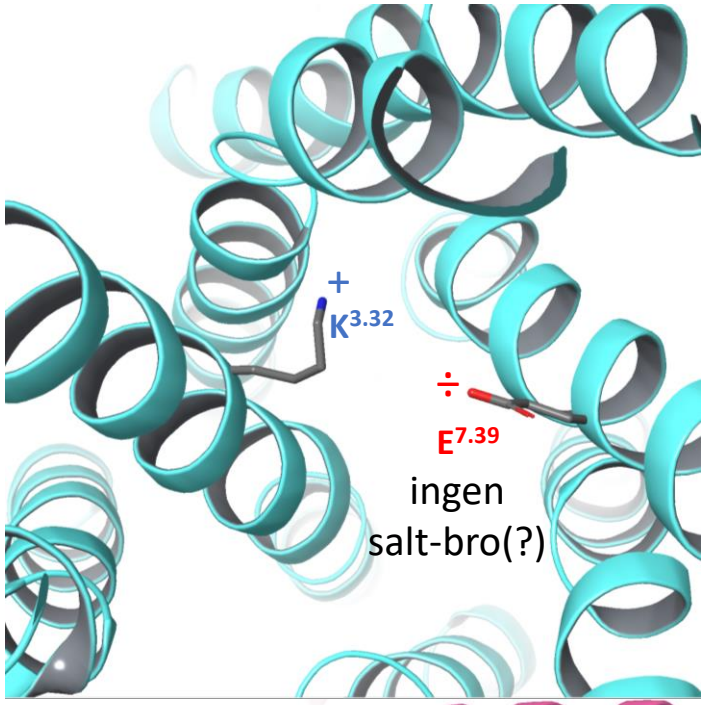


Bakgrunn

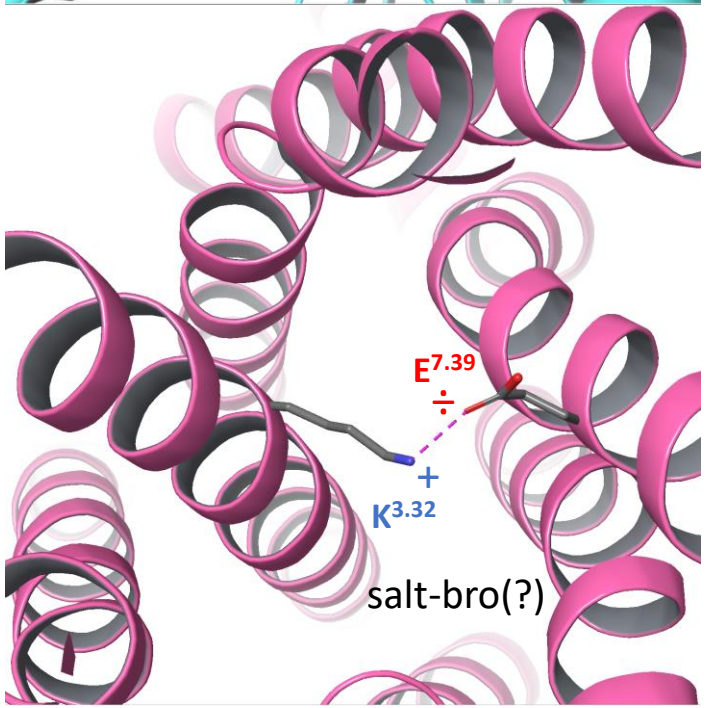
Analyse av sekvenser & strukturer



Receptor	Fire nøkkelposisjoner			
	Position			
	1.39	2.60	3.32	7.39
CCR1	Y	W	Y	E
CCR2	Y	W	Y	E
CCR3	Y	W	Y	E
CCR4	Y	W	Y	E
CCR5	Y	W	Y	E
CCR6	Y	W	Y	E
CCR7	Y	W	Y	Y
CCR8	Y	Q	Y	E
CCR9	Y	W	Y	Q
CCR10	S	A	Y	S
CXCR1	Y	W	K^+	$E^÷$
CXCR2	Y	W	K^+	$E^÷$
CXCR3	Y	W	F	S
CXCR4	Y	W	Y	E
CXCR5	Y	A	H	E
CXCR6	Y	W	Y	E
CX3CR1	Y	W	F	E
XCR1	Y	W	F	R
ACKR1	F	L	W	E
ACKR2	Y	W	Y	E
ACKR3	Y	W	F	Q
ACKR4	L	W	Y	E
CCRL2	C	W	Y	K
Consensus	**Y (83%)**	**W (83%)**	**Y (65%)**	**E (70%)**



CXCR2
Kjent 3D-struktur
(Liu, *Nature* 2020)

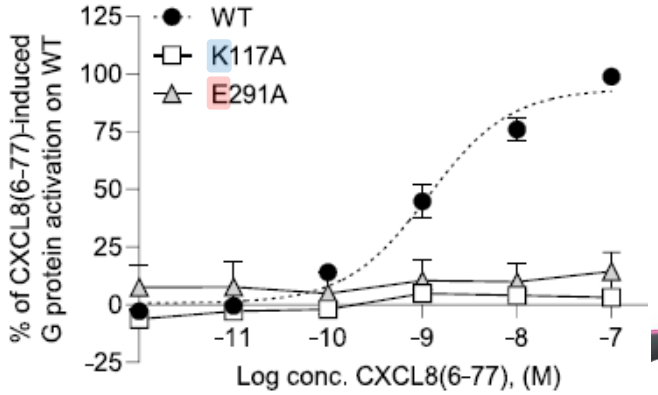


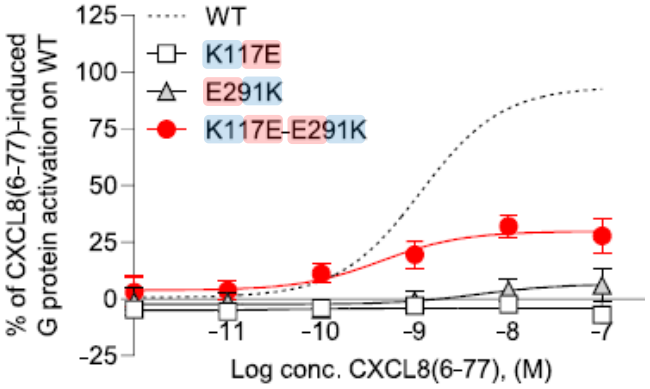
CXCR1
Ukjent 3D-struktur

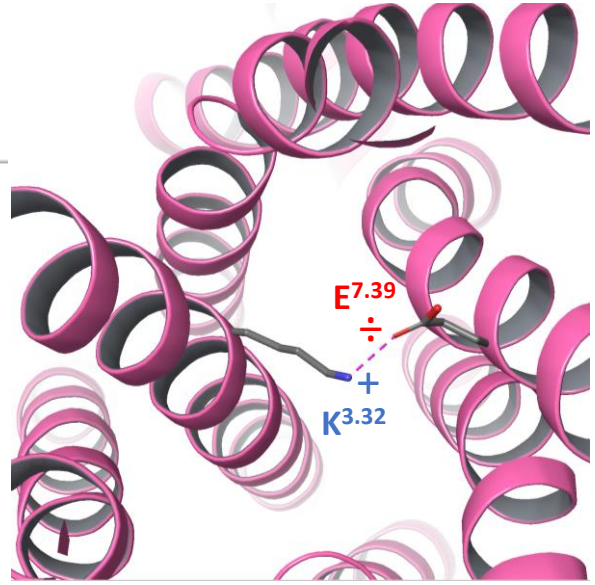
Her vises en *predikert* 3D-struktur (AlphaFold)

Resultater

CXCR1

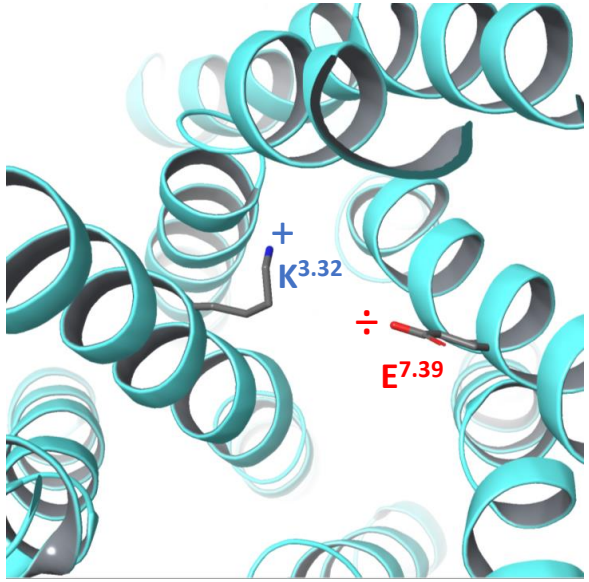




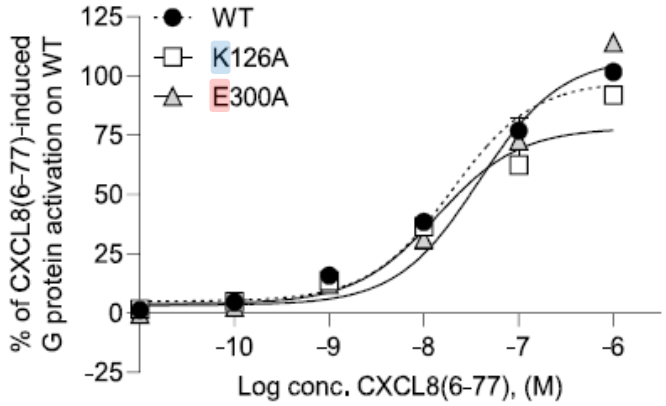


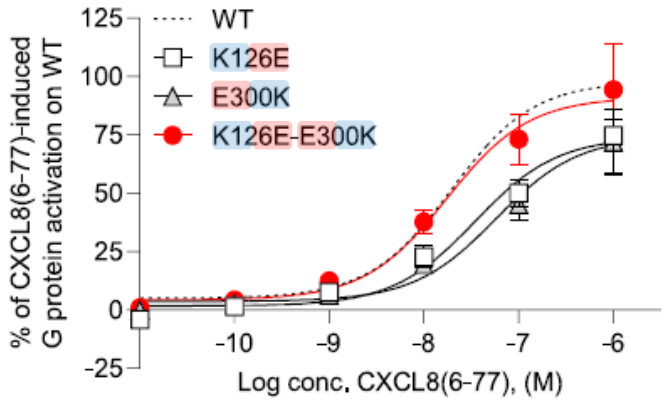
Sensitiv for endringer;
salt-bro viktig for funksjon

CXCR2



Robust mot endringer;
salt-bro <u>ikke</u> viktig for funksjon





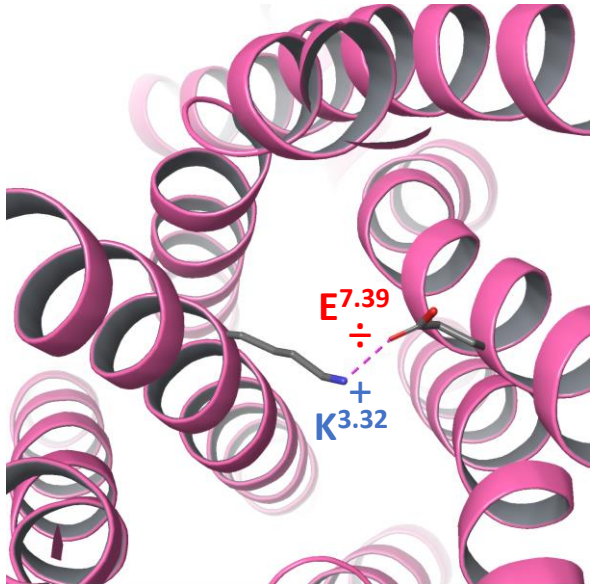
Konklusjon

Har identifisert en funksjonell forskjell mellom CXCR1 og CXCR2 som kan bidra til utvikling av mer spesifikke legemidler

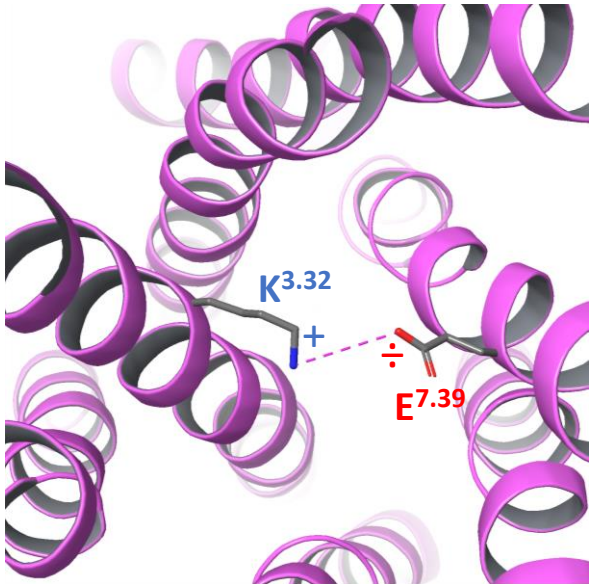
Uventet bekreftelse

- 14. juli 2023: Vår artikkel publisert
- **19. juli 2023**: 3D-struktur av CXCR1 publisert (Ishimototo, *Nature Comm.*); denne viser en **salt-bro mellom $K^{3.32}$ og $E^{7.39}$**, akkurat som vi skisserte i artikkelen:

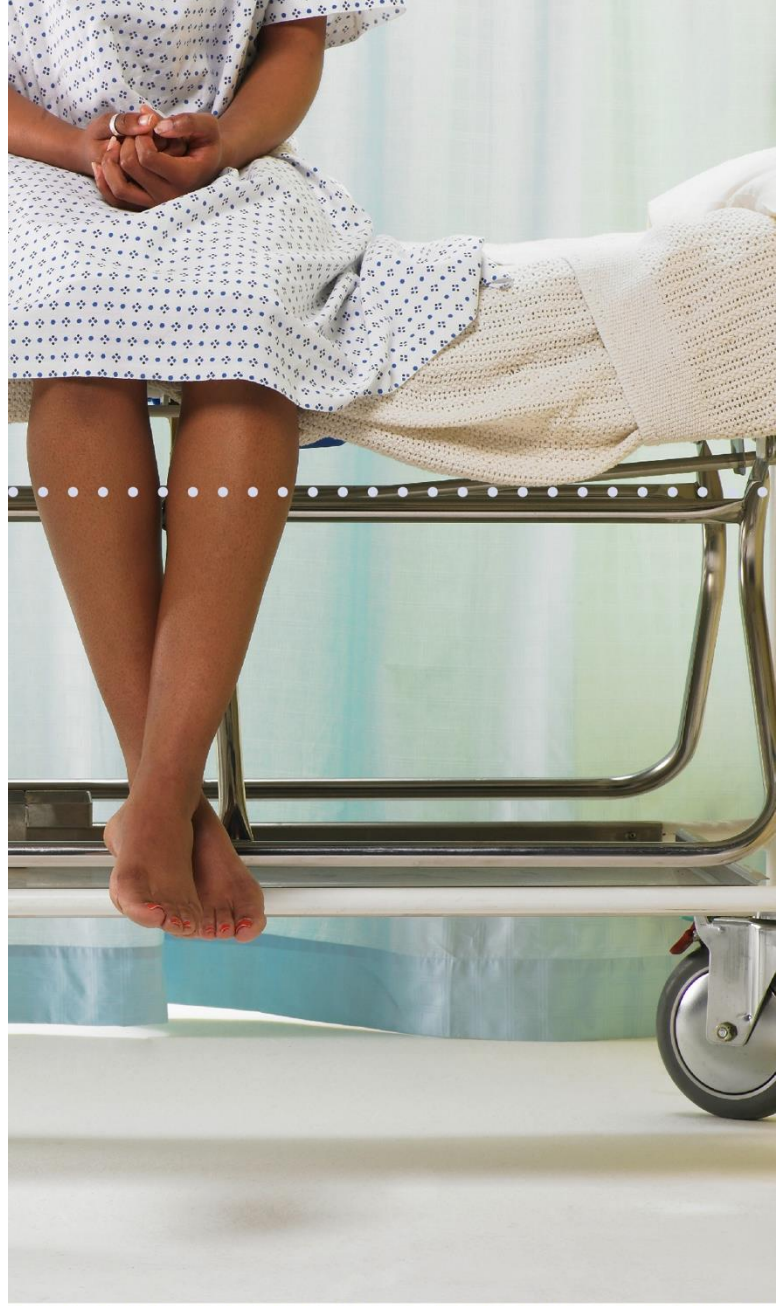
CXCR1 (predikert salt-bro)



CXCR1 (observert salt-bro)



Takk for oppmerksomheten!



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